determined by an anti-proliferative assay using hematological and solid tumor cell lines shows IC50 values as low as 54 nM. MV-4−11 leukemia cells treated with SGI-1776 shows a dramatic decrease in phospho-BAD levels (a direct substrate of the Pim kinases) as determined by western blot with an EC50 value of <10 nM. In conjunction with the anti-proliferative and phospho-BAD data, cell death via apoptosis was observed in cells treated with SGI-1776. Pharmacokinetic analysis of SGI-1776 in rats has demonstrated good oral bioavailability of the drug. Treatment of xenograft tumor models showed decrease tumor growth rates and in some models complete regression of the tumors at doses as low as 67 mg/kg. To determine if SGI-1776 was modulating Pim-1 activity in xenograft tumor in vivo pharmacodynamic studies were performed. Bad phosphorylation levels were determined in xenograft tumors by western blot and IHC. These studies showed that oral delivery of SGI-1776 modulated PD markers and could do so in a dose dependent manner. SuperGen's SGI-1776 exhibits potent inhibition of Pim kinase activity, translating into potent inhibition of cellular signaling pathways, cancer cell proliferation, and in vivo tumor progression in non-clinical models.

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Effectiveness of 6-(7-nitro-2,1,3-benzoxadiazol-4-ylthio)hexanol (NBDHEX) on human osteosarcoma and melanoma tumours

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Background: NBDHEX is a promising anticancer drug which activates the c-Jun N-terminal kinase (JNK) pathway through the binding to glutathione S-transferase P1−1 (GSTP1−1). The disruption of the GSTP1−1-JNK complex is followed by a remarkable pro-apoptotic effect in tumor cells. In the present work we assessed the in vitro and in vivo effectiveness of NBDHEX on poorly responsive/resistant human osteosarcoma (OS) and melanoma models.
Material and Methods: NBDHEX was tested on 10 human OS cell lines and on 20 U-2OS or Saos-2 variants resistant to cisplatin (CDDP), doxorubicin or methotrexate and on two human melanoma cell lines (A375 and Me501 cells). Apoptosis was evaluated by chromatin fragmentation and caspase activation tests. GSTP1−1 levels and activation of the JNK/cJun pathway were tested by Western blot. The activity of the NBDHEX-CDDP combination was evaluated in OS cell lines sequentially exposed to equitoxic concentrations of the two drugs. In vivo experiments were performed on SCID mice implanted with A375 or Me501 tumors. Mice were treated orally with different doses of NBDHEX. Tumor growth inhibition (TI) was monitored three times a week. Toxicity was evaluated on the basis of weight loss and the autopsy findings. The mitotic index (MI) was determined by microscopy.
Results: NBDHEX was very active on both OS and melanoma cells with IC50 values in the micromolar range, independently of GSTP1−1 levels. In these cell lines NBDHEX induced the activation of the JNK/cJun pathway and a strong pro-apoptotic effect. Drug combination studies on OS cells, showed that NBDHEX can be used in association with CDDP. Anti-tumor efficacy was observed in vivo against Me501 human melanoma. NBDHEX showed 70% TI after oral (daily ×25) administration. Tolerability was good at all tested doses with no significant changes in body weight. The tumor MI value was decreased by 50% after NBDHEX treatment. Similar results were obtained on advanced human melanoma, A375 model, with 63% TI after oral (daily ×10) treatments. Tests on in vivo efficacy of NBDHEX on OS models are still ongoing.
Conclusions: The low responsive/resistant OS and melanoma tumor cells are efficiently committed to death by NBDHEX. This drug is effective and well tolerated in in vivo tumor models. These findings indicate that activation of JNK/cJun pathway, through a selective GSTP1−1 targeting, could prove a promising new strategy for treating tumors that respond poorly to conventional therapies.

### 334   POSTER
Molecular modelling and synthesis of novel CYP26A1 inhibitors

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Cancer is one of the major causes of death in the world nowadays. The treatment of cancer could be accomplished through surgery or radiation

followed by application of chemotherapy, however the main drawback of most chemotherapeutic agents is the side effects which accompany their use, many of which arise owing to the inability of chemotherapeutic agents to differentiate between the malignant cells and the fast dividing normal cells. One of the major targets of the scientist is to find drugs which can affect cancer cells without impairing the normal cells. One approach to fulfill this criterion is through drugs which act upon Cytochrome P450, such as CYP19A1, CYP24A1 and CYP26A1.
Our main target is CYP26A1, which is the enzyme responsible for the metabolism of retinoic acid. Retinoic acid (RA), the active metabolite of vitamin A, binds with nuclear receptors RAR/RXR to regulate cell growth and differentiation in a variety of cell types, and can reverse malignant growth in vitro and in vivo. These properties have led to RA being used effectively in a number of clinical situations including APL and neuroblastoma. However, in both cases resistance to RA can limit the therapeutic benefits observed. Evidence is growing that this resistance is related to up-regulation of CYP26 resulting in accelerated metabolism of RA. Inhibition of CYP26A1 results in the increase of the tissue level of retinoic acid and/or maintenance of a high therapeutic level of retinoic acid. Liarozole is the most studied and first CYP26A1 inhibitor to undergo clinical investigation for the treatment of ichthyosis. Liarozole also showed promising results in the treatment of prostate cancer compared with cyproterone acetate, however it lacked CYP selectivity. A model for CYP26A1 enzyme, developed within our group, has been used for docking of our compounds. Also, we have generated a pharmacophore model for CYP26A1 inhibitors. The docked compounds have been synthesized using different chemical methods, and analyzed for their CYP26A1 inhibitory activity within our laboratory using MCF-7 breast cancer cells. The tested compounds have shown good to moderate activity against CYP26A1, with nanomolar to low micromolar activities and good selectivity for CYP26A1.
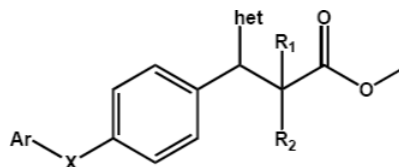
General structure formula of the tested compounds. Ar = naphthyl, phenyl; X = O, NH; R_1 = H, CH_3; R_2 = H, CH_3.

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Identification of potent, selective sphingosine-1-phosphate 1 receptor (S1P1R) antagonists with antitumor activity

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Background: Bioactive lipids, including sphingosine-1-phosphate (S1P), have been shown to play important roles as signaling and regulatory molecules. Many, if not all, of the effects of S1P are mediated by five distinct but related G-protein coupled receptors, S1P1−5. Despite the large body of evidence for the role of S1P1R in angiogenesis, proliferation, and cell migration, the development and application of potent, selective, small molecule S1P1R antagonists is lacking. The goal of this study was to identify and characterize selective S1P1R antagonists using in vitro and in vivo angiogenesis and tumor models.
Methods: High-throughput screening (HTS) of approximately 4.5 million compounds was conducted using HEK-293 cells overexpressing S1P1R and a modified cyclic nucleotide gated channel that served as a biosensor for intracellular cAMP. Compounds identified from screening were further optimized by medicinal chemistry and characterized using a variety of bioassays. Migration assays were conducted with SK-Hep-1 cells using a Boyden chamber assay. S1P1R selectivity assays were performed using β-arrestin recruitment assays in cell lines that expressed S1P1R, S1P2R or S1P3R. Tube formation assays were conducted using conditioned media from DU145 cells in a co-culture system consisting of primary human fibroblasts and endothelial cells. The effects of S1P1R antagonists were assessed in vivo using human tumor xenograft models in nude mice.
Results: Several potent, selective compounds were identified from HTS. Optimization by medicinal chemistry yielded S1P1R antagonists with in vitro IC50 values <10 nM in the cAMP biosensor assay that were highly selective for S1P1R compared to the related S1P2R and S1P3R receptors. Moreover, compounds were equally potent in inhibiting migration of SK-HEP-1 cells and blocking in vitro tube formation. Selected compounds had high oral bioavailability in rodents, and administration to nude mice bearing MDA-MB-231 tumor xenografts produced significant changes in tumor microvessel structure and evidence of antitumor activity.